

AMENDMENTS TO THE CLAIMS

This listing shows the claims as pending. No amendments are made herein.

1. (withdrawn) An isolated, non-canonical zinc finger binding protein encoded by the polynucleotide of claim 30.

2-22. (canceled)

23. (withdrawn) The isolated polynucleotide of claim 30, wherein the target sequence is in an animal cell.

24. (withdrawn) The isolated polynucleotide of claim 23, wherein the target sequence is in a human cell.

25. (previously presented) The isolated polynucleotide of claim 30, wherein the target sequence is a promoter sequence.

26. (previously presented) The isolated polynucleotide of claim 30, wherein the zinc finger binding protein comprises three zinc finger components.

27. (previously presented) The isolated polynucleotide of claim 30, wherein the target sequence comprises about 9 to about 14 contiguous base pairs.

28. (previously presented) The isolated polynucleotide of claim 26, wherein the third zinc finger component comprises a non-canonical zinc finger component.

29. (cancelled)

30. (currently amended) An isolated polynucleotide encoding a non-naturally-occurring zinc-finger binding protein comprising a non-canonical zinc finger component, wherein:

(i) said non-canonical zinc finger component contains a beta turn comprising two amino-terminal zinc coordinating cysteine or histidine residues and an alpha helix comprising two carboxy-terminal zinc coordinating cysteine or histidine residues, wherein at least one of the zinc coordinating residues is a histidine residue and at least one of the zinc coordinating residues is a cysteine residue;

(ii) the non-canonical zinc finger component comprises 1, 2, 3, 4, 6 or 7 amino acids between the two carboxy-terminal zinc coordinating residues and 2, 3 or 4 amino acids between the two amino-terminal zinc coordinating residues, wherein if there are 3 amino acid residues in the region between the two carboxy-terminal zinc coordinating residues, at least one of the residues in this region is altered as compared to a naturally-occurring zinc finger with 3 residues in the region between the two carboxy-terminal zinc coordinating residues; and

(iii) the non-canonical zinc-finger binding domain protein comprises a recognition helix of at least 7 amino acids in length, wherein the recognition helix is non-naturally occurring and is engineered to bind to a target nucleic acid sequence in a plant cell.

31. (original) An expression vector comprising the polynucleotide of claim 30.

32. (previously presented) An isolated host cell comprising the polynucleotide of claim 30.

33. (withdrawn) A fusion polypeptide comprising: (a) an isolated zinc finger binding protein according to claim 1 and (b) at least one functional domain.

34. (withdrawn) The polynucleotide of claim 39, wherein the functional domain is a repressive domain.

35. (withdrawn) The polynucleotide of claim 34, wherein the repressive domain is selected from the group consisting of KRAB, MBD-2B, v-ErbA, MBD3, TR and members of the DNMT family.

36. (previously presented) The polynucleotide of claim 39, wherein the functional domain is an activation domain.

37. (previously presented) The polynucleotide of claim 36, wherein the activation domain is selected from the group consisting of maize C1, VP16, p65 subunit of NF-kappa B, and VP64.

38. (withdrawn) The polynucleotide of claim 39, wherein the functional domain is an endonuclease.

39. (previously presented) An isolated polynucleotide according to claim 30 further encoding a functional domain.

40. (original) An expression vector comprising the polynucleotide of claim 39.

41. (previously presented) An isolated host cell comprising the polynucleotide of claim 39.

42. (withdrawn) A method of modulating expression of a gene in a plant cell, the method comprising the step of contacting a cell with a polynucleotide according to claim 39.

43. (withdrawn) The method of claim 42, wherein the zinc finger binding protein binds to a target site in a gene encoding a product selected from the group consisting of

gamma-tocopherol methyl transferase (GMT), vascular endothelial growth factor, erythropoietin, androgen receptor, PPAR- γ 2, p16, p53, pRb, dystrophin and e-cadherin.

44. (withdrawn) The method of claim 42, wherein the functional domain comprises a repressive domain.

45. (withdrawn) The method of claim 44, wherein the repressive domain is selected from the group consisting of KRAB, MBD-2B, v-ErbA, MBD3, TR and members of the DNMT family.

46. (withdrawn) The method of claim 42, wherein the functional domain comprises an activation domain.

47. (withdrawn) The method of claim 46, wherein the activation domain is selected from the group consisting of maize C1, VP16, p65 subunit of NF-kappa B, and VP64.

48. (withdrawn) The method of claim 42, wherein the functional domain is an endonuclease.

49 to 51. (canceled).

52. (withdrawn) A composition comprising a non-naturally-occurring zinc-finger binding protein according to claim 1 and a pharmaceutically acceptable excipient.

53. (previously presented) A composition comprising a polynucleotide according to claim 39 and a pharmaceutically acceptable excipient.

54. (previously presented) The isolated polynucleotide of claim 26, wherein the first zinc finger component comprises a non-canonical zinc finger component.

55. (previously presented) The isolated polynucleotide of claim 30, wherein the zinc finger binding protein comprises four zinc finger components.

56. (previously presented) An isolated polynucleotide encoding a non-naturally occurring zinc-finger binding protein comprising a non-canonical zinc finger component, wherein:

(i) said non-canonical zinc finger component contains a beta turn comprising two amino-terminal zinc coordinating cysteine and an alpha helix comprising two carboxy-terminal zinc coordinating cysteine or histidine residues, wherein one of the carboxy-terminal zinc coordinating residues is a histidine residue and one of the carboxy-terminal zinc coordinating residues is a cysteine residue;

(ii) the non-canonical zinc finger component comprises 2 amino acids between the two amino-terminal zinc coordinating cysteine residues; and

(iii) the protein comprises a non-naturally occurring recognition helix that is engineered to bind to a target nucleic acid sequence.

57. (previously presented) The polynucleotide of claim 56, wherein the carboxy-terminal zinc coordinating histidine residue is amino terminal to the carboxy-terminal zinc coordinating cysteine residue.

58 to 61. (cancelled).

62. (new): An isolated polynucleotide encoding a non-naturally-occurring zinc-finger binding protein comprising a non-canonical zinc finger component comprising a non-canonical zinc finger comprising the sequence C-(X^A)₂₋₄.C-X^B-(K/R)-X^C-F-X^D-X^E-X^F-X^G-X^H-X^I-X^J-X^K-H-(X^L)₁₋₇-H (SEQ ID NO:2), where X is any amino acid, wherein:

(i) the C and H residues are zinc coordinating residues and at least one of the C residues is replaced with a H residue or at least one of the H residues is replaced with a C residue such that at least one of the zinc coordinating residues is a histidine residue and at least one of the zinc coordinating residues is a cysteine residue;

(ii) the non-canonical zinc-finger comprises a recognition helix of at least 7 amino acids in length (X^B through X^K), wherein the recognition helix is non-naturally occurring and is engineered to bind to a target nucleic acid sequence; and

(iii) the non-canonical zinc finger comprises at least one amino acid modification selected from the group consisting of substitution of one or more of the amino acid residues at X^B, X^C or X^D; substitution of one or more of the amino acid residues at X^L; addition of four amino acid residues C-terminal to the carboxy-terminal zinc coordinating residue of the non-canonical zinc finger; and combinations thereof.

63. (new) The isolated polynucleotide of claim 62 wherein the target nucleotide sequence is in a plant cell.

64. (new) The isolated polynucleotide of claim 30 wherein the target nucleotide sequence is in a plant cell.